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SYMPATRIC SONG VARIANT IN MOUNTAIN CHICKADEES (*POECILE GAMBELI*) DOES NOT REDUCE AGGRESSION FROM BLACK-CAPPED CHICKADEES (*POECILE ATRICAPILLUS*)

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by

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ABSTRACT

Various species interact with one another daily and, when habitats overlap and species compete for resources, the interactions are often negative. Character displacements are shifts in traits that typically occur in regions of geographic overlap of closely related species. These shifts, which act to reduce negative interactions, can be behavioural, social or morphological. Previous research has shown that mountain chickadees (*Poecile gambeli*) have an altered song structure in regions of geographic overlap with the more dominant black-capped chickadee (*Poecile atricapillus).* Similar to the situation for European and Asian tits, mountain chickadees may have changed their song to decrease aggression from black-cappeds. To test this hypothesis, I conducted a playback study with black-capped chickadees in Prince George, BC in which I observed how they responded to the songs of mountain chickadees recorded in regions where the two species were (1) sympatric and (2) allopatric. I used principal component analysis to collapse behavioural response variables into a single "approach" variable and a single "vocalization" variable. I then used mixed-model analysis to determine whether there was a difference in approach or vocalization response to the two types of mountain chickadee songs. Black-capped chickadees responded with equal intensity to both types of mountain chickadee songs. My results demonstrate that mountain chickadee songs with the sympatric song variant do not reduce heterospecific aggression from black-capped chickadees. To my knowledge, this is the only instance of a character shift unassociated with reduced aggression in the family Paridae and raises interesting questions about the selective pressures leading to the evolution of this song divergence. It is possible that

different selective pressures may result in similar evolutionary outcomes in the form of altered songs in sympatric populations.

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INTRODUCTION

When ranges overlap and species compete for resources, negative interactions frequently occur. Closely related species often inhabit the same ecological niche and have similar life history traits, which can lead to high levels of competition between them. One way of reducing these negative interactions is through character displacement (Brown & Wilson 1956). Character displacements involve divergences in ecological, behavioural, morphological, or physiological traits between closely related species in regions of geographic overlap, which acts to differentiate closely related competitors and thus reduce interspecific competition (Brown & Wilson 1956). Character displacement has been demonstrated across a range of several taxa including several species in the order Carnivora (Davies et al. 2007) as well as salamanders (Adams 2004), lizards (Huey 1974; Melville 2002), fish (Schluter & McPhail 1990) and birds (Grant 1972; Doutrelant et al. 2000; Grava et al. 2013; Hamao et al. 2015).

In carnivores, carnassial teeth are an integral part of food processing and the length of these teeth can vary based on the prey items being consumed. Davies et al. (2007) used a phylogenetic analysis with complete species level sampling for Carnivora, and examined all sister species pairs, excluding marine taxa and domestic dogs and cats. In sister species for which there is minimal geographical overlap, carnassial teeth are very similar in length. However, sister species with the greatest geographical overlap show the most differentiation in carnassial length. Interspecific competition for food resources between sister species in sympatry is suggested as the cause of this morphological character displacement; divergent carnassial tooth length has been selected for (Davies et al. 2007).

In a well-studied terrestrial salamander genus, *Plethodon*, aggressive behavior was directly associated with morphological divergence in both *P. jordani* and *P. teyahalee.* In the Southern Appalachian Mountains of the Eastern United States, *P. teyahalee* inhabits lower elevations, whereas *P. jordani* inhabits higher elevations. However, there is a naturally occurring region of range overlap where frequent aggressive interactions such as biting, occur. Head shape differs in both allopatric and sympatric populations of *P. jordani* and *P. teyahalee*, but allopatric populations have less morphological divergence than sympatric populations (Adams 2004).

Two subterranean skink species, *Typhlosaurus gariepensis* and *T. lineatus*, occur sympatrically in the Kalahari Desert, with the smaller *T. gariepensis* living entirely within the range of the larger *T. lineatus*. In this area, *T. lineatus* have larger snout-vent lengths, head lengths, and head dimensions than they do in areas where their range does not overlap with that of allopatric *T. lineatus.* Based on dietary and morphological evidence, female and immature *T. lineatus* have undergone both a morphological and ecological character displacement that reduces dietary overlap with sympatric *T. gariepensis*; adult male *T. lineatus*, however, have not (Huey 1974). Rather than switching prey taxa altogether, as immature and female *T. lineatus* have done, *T. lineatus* males switch within the same prey taxon. Ultimately, this does not result in less diet overlap with *T. gariepensis* compared to the reduction in overlap that has occurred for females and immatures; nonetheless, it is a unique behavioural change occurring only in sympatry (Huey 1974).

Similarly, lizard species, *Niveoscincus microlepidotus* and *N. greeni,* which occur in alpine areas, can inhabit two distinct habitat types: boulder fields and alpine heaths. Although alpine heaths have fewer thermal opportunities (i.e., fewer basking sites) than boulder fields- both species can use either type of habitat. In allopatry, *N. microlepidotus*, which is smaller and

subordinate to *N. greeni*, was found significantly more often in boulder habitats than alpine heaths, whereas in sympatry it is restricted to the latter habitat type. Also, while in sympatry with the dominant *N. greeni*, *N. microlepidotus* exhibits a reduced body size, indicating both a morphological and ecological character displacement (Melville 2002).

This pattern has also been noted in two subspecies of marine three-spined sticklebacks (*Gasterosteus aculeatus*), which exhibit ecological character displacement when in sympatry with closely related subspecies. Only one subspecies of three-spined sticklebacks typically occurs in an area, however, pairs of subspecies have been found to co-occur in the Strait of Georgia, within four drainages around three islands. When isolated from each other, both subspecies can inhabit the limnetic and benthic zones in a lake. In sympatry, however, one subspecies inhabits the limnetic zone and the other remains in the benthic zone: the two occupy separate niches. Distributions of the three-spined sticklebacks to either limnetic or benthic zones suggest that these subspecies pairs evolved together several times (Schluter & McPhail 1992).

Character displacements have occurred in a range of avian species. Studies on closely related species pairs, such as great tits (*Parus major*) and Eurasian blue tits (*Cyanistes caeruleus)*, mountain chickadees (*Poecile gambeli*) and black-capped chickadees (*Poecile atricapillus*), and varied tits (*Poecile varius*) and Japanese tits (*Parus minor*) all found that when such species lived in sympatry, the subordinate species diverged in expression of shared traits from the sister species, undergoing a character displacement (Doutrelant et al. 2000; Grava et al. 2013; Hamao et al. 2015). For example, Eurasian blue tits that inhabit regions where few or no dominant great tits are present produce songs that are similar to those of great tits (Doutrelant et al 2000). In regions of sympatry, however, Eurasian blue tits, which are the subordinate species, add a trill to the end of their song. Great tits react less strongly to the altered Eurasian blue tit trilled songs

than they do to either untrilled Eurasian blue tit song or the song of conspecific great tits; the character shift in Eurasian blue tit song acts to reduce aggression from great tits (Doutrelant et al. 2000). A study by Hamao et al. (2015) of Japanese tits and varied tits also shows this pattern. In regions where the two species live in sympatry, subordinate Japanese tits sing at a lower frequency, acoustically diverging from the songs of varied tits, as well as from the songs of Japanese tits living where varied tits are not present. These results suggest that the subordinate Japanese tit alters its song characteristics to avoid harassment by the dominant varied tit (Hamao et al. 2015); although future playback studies are needed to confirm that this results in reduced aggression.

In North America, the black-capped chickadee is socially dominant to the mountain chickadee (Grava et al. 2012a; Grava et al. 2012b; Grava et al. 2013). Typically, the two species segregate due to different habitat preferences and elevation, but historically overlapping zones exist throughout their ranges (Figure 1). With the onset of forestry practices that create habitat mosaics that do not occur naturally as often, the two species now frequently overlap (Grava et al. 2012a).

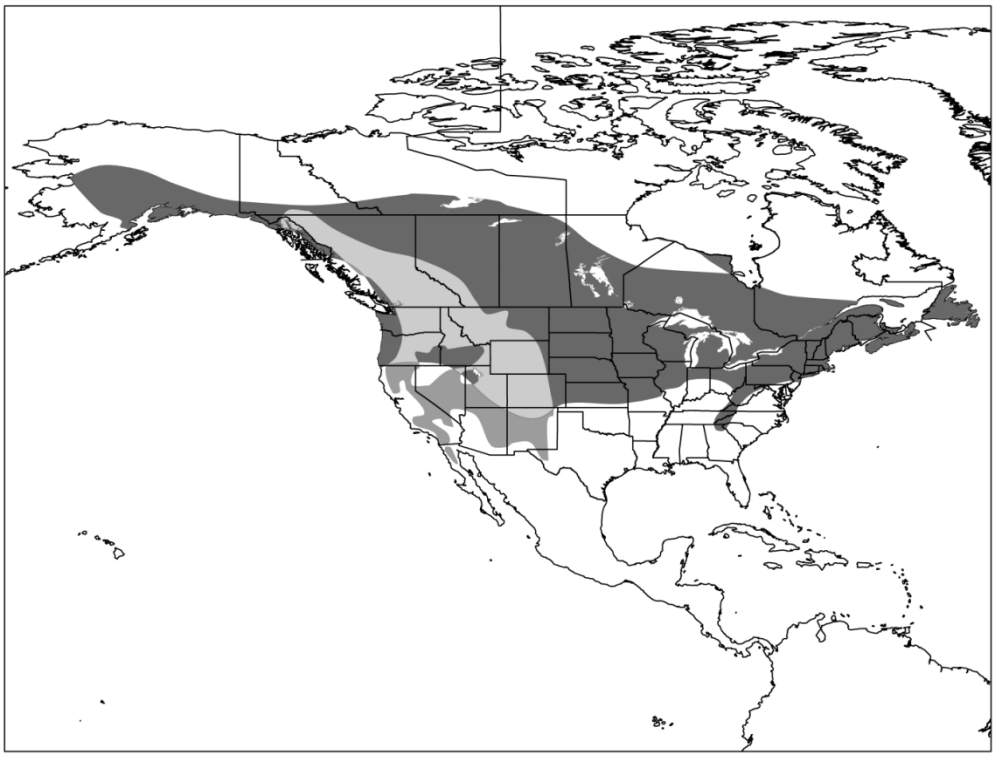

Figure 1. Map of North America showing black-capped chickadee range (dark grey), mountain chickadee range (medium grey) and the zone in which both species occur (light grey). Data obtained from Bird Life International (Birdlife International 2015).

Mountain chickadees are subordinate to black-capped chickadees and exhibit greater range-wide variation in their song (Lohr 2008), which may be driven by dominance-mediated character displacement. Grava et al. (2013) found differences in mountain chickadee song structure between regions of co-occurrence and isolation, whereas black-capped chickadees have a consistent song throughout North America. In areas of contact between mountain chickadees and black-capped chickadees, the mountain chickadee song would shift away from the structure of the black-capped song in some way.

While research by Grava et al. (2012a; 2013) suggests that mountain chickadees add a song variant in areas of sympatry with black-capped chickadees, no studies have examined whether

these sympatric song variants result in fewer negative interactions with dominant black-capped chickadees, as seen in European and Asian tits (Doutrelant et al. 2000; Hamao et al. 2015). Following the study design of Doutrelant (2000), which examined the behavioural response of great tits to Eurasian blue tit vocalizations recorded in areas of sympatry and allopatry, I tested the hypothesis that mountain chickadees alter their vocalizations to minimize negative interactions from black-capped chickadees. I predicted that black-capped chickadees would respond less aggressively to mountain chickadee songs recorded in areas of sympatry (sympatric song variant) than to allopatric mountain chickadee songs (allopatric song variant).

MATERIALS AND METHODS

Playback Construction

Conforming to playback guidelines outlined by McGregor (1992; 2000) and McGregor et al. (1992), I created a series of 10 playback dyads (paired playbacks, one playback with sympatric songs and one with allopatric songs) using mountain chickadee songs from populations across British Columbia and the United States. Stimuli were obtained from the study by Grava et al. (2013). “Allopatric” playbacks consisted of mountain chickadee songs taken from a region in which black-capped chickadees were not present. “Sympatric” playbacks consisted of mountain chickadee songs recorded from areas where both chickadee species co-exist. Each playback consisted of 12 songs per minute spaced approximately 5 to 6 seconds apart, for a total of 24 songs over a total of 2 minutes (Figure 2). The playbacks were created using Avisoft SASLab Pro software (Specht 2012), which allowed me to standardize the volume and amplitude of all songs. I used Audacity software (Audacity Team 2008) to select a series of 3-4 different songs used to create one stimuli type, either allopatric or sympatric. These selected songs were

then randomly mixed and repeated within each playback for variation, while still representing successive songs representing a single stimulus male. All songs used within a playback had an amplitude ranging between -21 and -18dB, resulting in a playback of either all allopatric songs or all sympatric songs. Low levels of Brownian noise were added to the playbacks to sound more natural and to diminish any noises from the editing process. The final stimuli included a 30 s blank intro followed by two minutes of stimulus broadcast. Unique dyads were created by randomly pairing one "allopatric" stimulus with a "sympatric" stimulus. I tested each dyad to ensure a sound level of 75dB during playback. All dyads were loaded onto an Apple iPod Touch for field playback and broadcast through a Logitech X100 Bluetooth speaker.

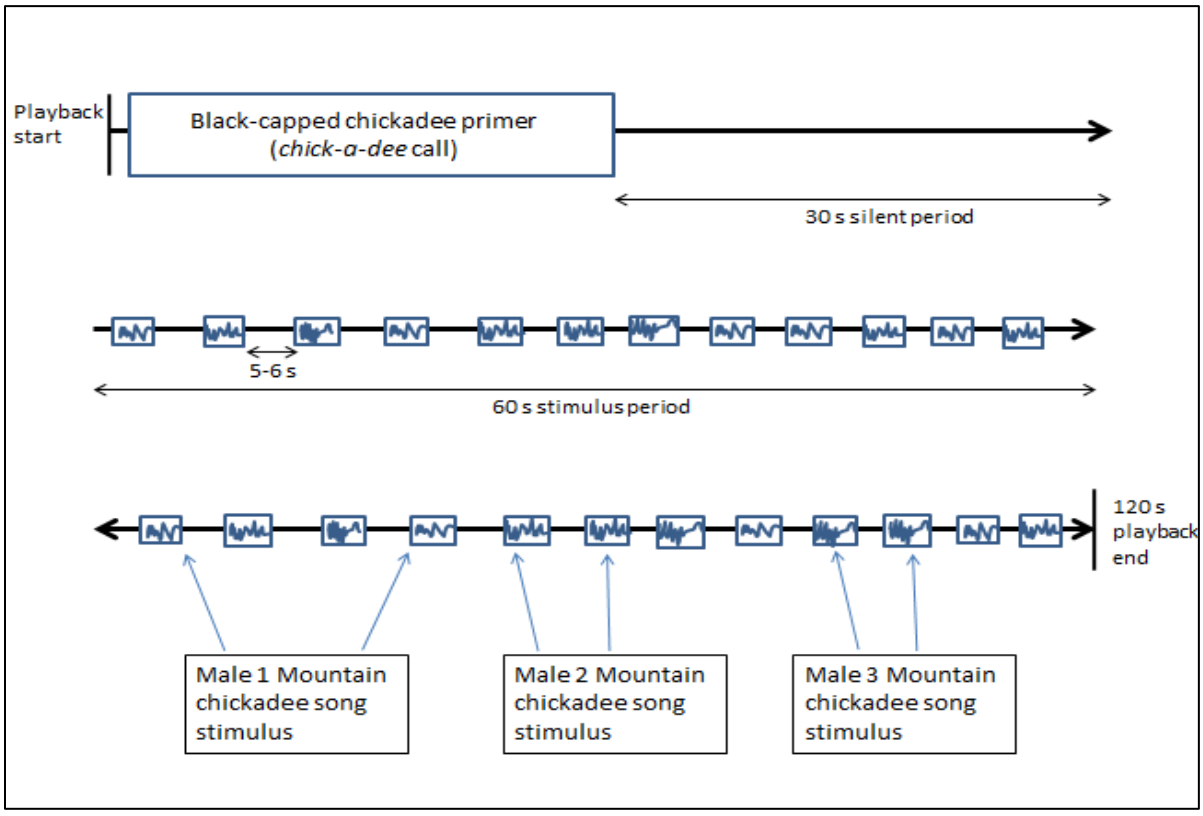

Figure 2. A diagram showing the construction of each playback audio file. Every song in each individual playback is either allopatric or sympatric, and is a combination of 3-4 mountain chickadee songs from various populations.

Field Methods

Playback experiments were conducted in Prince George, B.C. (53°54'40.1"N 122°45'18.6"W), from the 21-29th of April, 2015, following the methods of Doutrelant et al. (2000). I conducted playback experiments on a total of 22 black-capped chickadees, at 22 separated locations around the University of Northern British Columbia campus, the outskirts of Prince George and within the Prince George city park, Forests for the World. Playbacks were performed in the morning (07:00 to 12:00). To prevent interference, I chose focal males if they were in a territory alone (and/or with a mate) with no competing males in the vicinity, and if the bird was at least 250m from any previous birds tested that same day. Two to four 10m long ropes, with 5m markers, were set up equal distances apart radiating out from the middle of each focal bird's location, with a Logitech X100 Bluetooth speaker hanging in the centre, approximately 1m off the ground (Figure 3). The speaker faced the direction of the focal bird, and was connected via Bluetooth or using a plug-in adapter directly to the iPod.

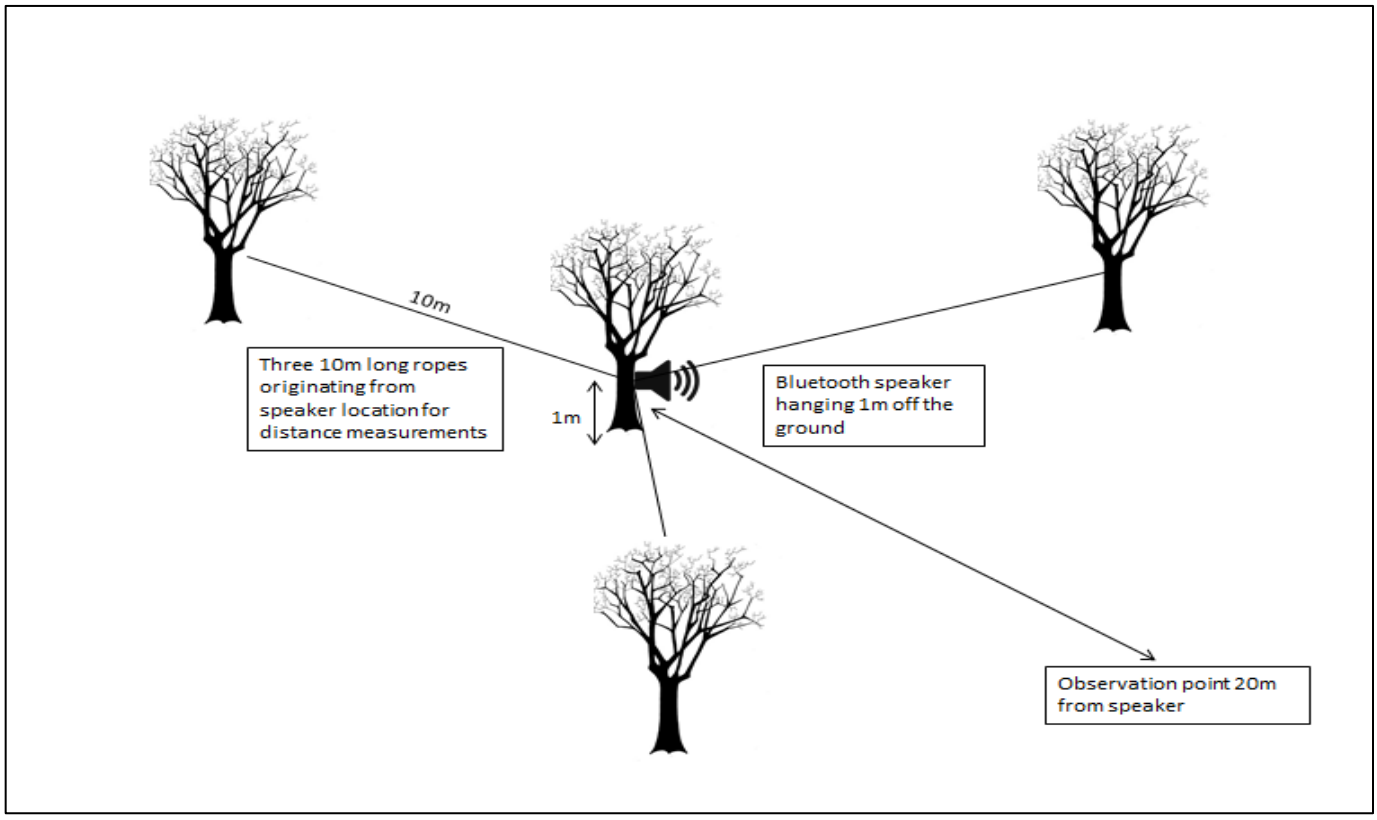

Figure 3. Diagram of the typical playback set-up. All playback trials were set-up based on these parameters, using two to four 10m ropes for distance estimates.

Each focal chickadee was given a randomly selected playback stimulus (either allopatric or sympatric), followed by a second playback stimulus of the alternative type 1-2 hours later at the same location, resulting in a total of two behavioural recordings per bird. The next focal bird was given a set of playback stimuli in the opposite order to randomize trials and avoid playback bias resulting from order effects. In each playback trial, focal birds were first called in with a "primer" call (a short playback of mobbing *chick-a-dee* calls), and were then given the selected playback recording. Behavioural responses were observed and recorded vocally using an Audiotechnica AT8015 microphone and a Marantz PMD661 MKII Professional Portable Flash Field Recorder. I verbally recorded the focal male's distance from the speaker, all short and long

flights taken, whether there was a mate present, vocalizations made (gargles, calls, or songs), and potential interruptions (e.g., intruding male, human disturbances such as construction, dogs, etc.).

Audio Analysis

Recordings were individually annotated using Avisoft SASLab Pro bioacoustics software, taking note of focal bird location and type of responses. These annotations were then exported into text files. The observations noted in the text files were processed and summarized using R statistical software (R Development 2014). Specifically, I extracted information on the focal male's distance from the speaker playback, time spent at each distance, how many vocalizations were made and level of aggressiveness with relation to the stimuli they were exposed to (whether the focal male song was non-overlapping, overlapping, or overlapped in relation to the playback stimuli), and time spent within 10 m of the speaker after the playback ended. Only birds with complete recording files of the trial were used in analysis; incomplete recordings were excluded from analysis ($n_{incomplete} = 2$).

Statistical Analysis

I used R statistical software to conduct iterative principal component analysis to collapse the variables into two holistic measures, one describing approach behavior and a second describing vocal response. Next, I constructed generalized mixed models in JMP 12 (SAS Institute 2015) to determine whether approach response or vocal responses varied with respect to the playback presented (allopatric/sympatric). In addition, I included playback order and starting distance (as well as an interaction term) as covariates. Male ID was included as a random factor to account for the paired design.

RESULTS

Principal Component Analysis

I collected 65 variables for each trial, which were associated with both individual approach and vocal responses to playback (Table A1). To collapse these variables, we conducted an iterative principal component analysis, using a broken stick approach to find principal components that best explained the data. Because many of the distance variables were related, we collapsed several of these variables (e.g., distance from 0-5m and distance from 5-10m were collapsed to distance from 0-10m) to derive a total of six variables, for which the first two principal components explained 70% of the variance (Table 1). PC1 (hereafter called "approach response") was most strongly associated with variables related to focal male distance from speaker during the playback period, and the association was negative (i.e., lower PC1 values indicate that birds approached more closely [minimum distance], spent more time close to the speaker [time 0-10m], spend less time far [time >20m], with a weak effect of singing fewer overlapping songs). PC2 (hereafter called "song response") was most strongly associated with variables related to focal male vocalization responses to the stimuli during the playback period (i.e., spending less time far [time >20m], singing and overlapping songs more; Table 1).

Table 1. Principal component analysis results of measures of distance from the speaker and song response variables. PC1 was most strongly associated with the approach variables, while PC2 was most associated with vocal responses. Bolded values reflect variables with contributions of greater than 0.33 or less than -0.33, which are considered to make a substantial contribution to the axis (Ho 2006).

Variable	PC1 Factor Loading	PC2 Factor Loading
Minimum distance to speaker	**-0.55**	0.21
Distance 0-10m	**0.55**	-0.08
Distance 10-20m	-0.24	-0.24
Distance >20m	**-0.45**	**0.36**
Total number of song responses	-0.12	**-0.69**
Total overlapping song responses	**-0.34**	**-0.54**
Total Variance explained	0.44	0.26

Approach

I analyzed paired responses from 20 individuals (40 observations total). Black-capped chickadees did not differ in approach response when presented with allopatric vs. sympatric mountain chickadee song (t_{19} = -0.58, p = 0.57). Because the approach response may have been influenced by the initial starting location of the black-capped chickadee, we included starting distance as a covariate and an interaction between starting distance and playback in the model. As anticipated, starting distance had a strong effect, but there remained no effect of playback type, nor was there an interaction between starting distance and playback type (starting distance: t_{17} = 7.02, p < 0.0001; playback type: t_{17} = 1.00, p = 0.33; starting distance*playback type: t_{17} = -1.31, p = 0.21). Because the interaction term was not significant, it was removed from the model.

Starting distance remained significant, but there was no significant effect of playback type (starting distance: $t_{18} = 7.88$, $p < 0.0001$; playback type: $t_{18} = -0.17$, $p = 0.87$; Figure 4).

Next, we generated a model with an additional effect of playback order (i.e., whether allopatric was presented first or second) and an interaction term to determine whether there was an order effect (i.e., whether the song played first [allopatric or sympatric] influenced the black-capped chickadee responses). Neither the interaction term nor the order effect were significant (first trial: $t_{18} = 0.62$, $p = 0.54$; playback type: $t_{18} = -0.06$, $p = 0.96$; first trial*playback type: $t_{18} = -0.49$, $p = 0.63$). Upon removal of the interaction term, there was still no effect of either playback order or playback type (first trial: $t_{18} = 0.44$, $p = 0.67$; playback type: $t_{19} = -0.58$, $p = 0.57$). When we included starting distance in the model, there was a significant effect of starting distance, however playback trial and order were not significant (first trial: $t_{18} = 0.51$, $p = 0.62$; playback type: $t_{18} = -0.17$, $p = 0.87$; starting distance: $t_{18} = 7.77$, $p = < 0.0001$).

Song

We found no difference in song response to the type of playback presented to black-capped chickadees (allopatric vs. sympatric; $t_{19} = -0.93$, $p = 0.36$). As with approach response, we included starting distance as a covariate and an interaction between starting distance and playback type in the model. There was no effect of starting distance, playback type, or the interaction term (starting distance: $t_{17} = -0.06$, $p = 0.95$; playback type: $t = 0.55$, $p = 0.59$; playback type*starting distance: $t_{17} = -1.33$, $p = 0.20$). Consequently, the interaction term was removed from the model. There remained no effect of either starting distance or playback type (starting distance: $t_{18} = -1.14$, $p = 0.26$; playback type: $t_{18} = -1.01$, $p = 0.33$; Figure 4).

Next, we produced models that included playback order and an interaction term. Playback order, playback type, and the interaction term were all not significant (first trial: t_{18} = -0.69, p = 0.50; playback type: t_{18} = -0.92, p = 0.37; first trial*starting distance: t_{18} = 0.39, p = 0.70), and remained non-significant after removal of the interaction term (first trial: t_{18} = -0.60, p = 0.56; playback type: t_{19} = -0.93, p = 0.37). An additional model was created to examine playback order as well as starting distance, however, there were still no significant effects (first trial: t_{18} = -0.55, p = 0.60; playback type: t_{18} = -1.00, p = 0.33; starting distance: t_{18} = -1.10, p = 0.29).

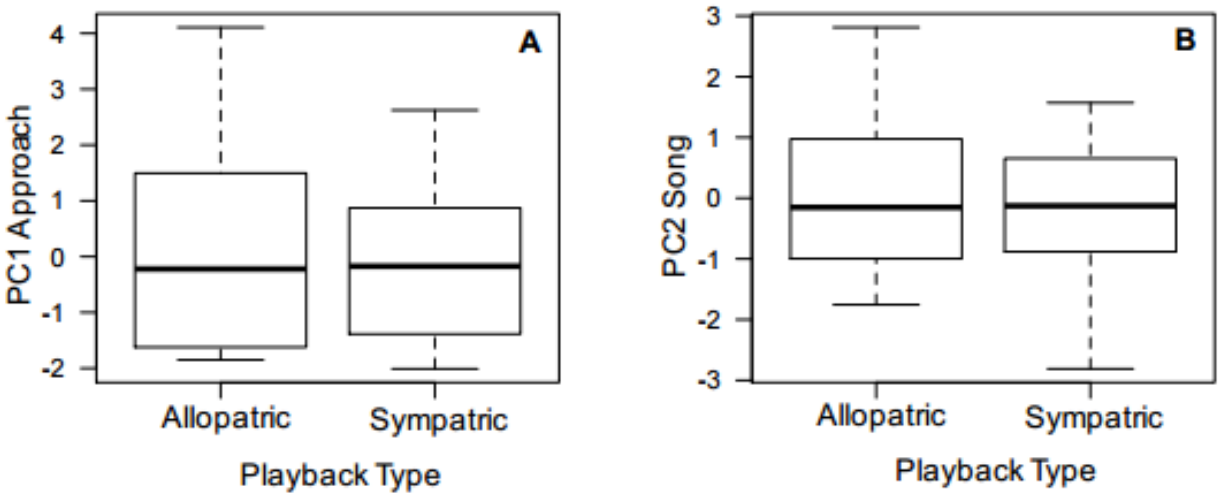

Figure 4. Analysis of response to allopatric vs. sympatric playback. Black-capped chickadee PC1 approach (A) or PC2 song (B) did not differ between allopatric and sympatric trials.

DISCUSSION

In contrast to studies on European and Asian tits, I did not observe that the song variant of subordinate mountain chickadees in regions of sympatry reduced aggressive responses from black-capped chickadees. Black-capped chickadees did not differentiate between sympatric and

allopatric mountain chickadee songs in any of their behavioural responses. During both sympatric and allopatric playback trials, black-capped chickadees reacted by either approaching the speaker or vocalizing during the trial, suggesting that black-capped chickadees recognize both song types of mountain chickadees, and respond to them as a potential threat. The sympatric song variant, or character shift, exhibited by mountain chickadees in populations where the two species are sympatric, did not appear to minimize negative interactions with black-capped chickadees. This result is unexpected due to the findings of studies on pairs of tit species (which are closely related to chickadees) in which the subordinate species do display character shifts that reduce negative interactions with the more dominant form (Doutrelant et al. 2000; Hamao et al. 2015).

Black-capped chickadees respond less aggressively to heterospecific calls than to conspecific calls (Grava et al. 2012b). However, when I presented black-capped chickadees with only heterospecific mountain chickadee songs, there was an equal reaction to both the allopatric and sympatric versions of this song. One possibility is that the sympatric populations of mountain chickadees are in the early stages of character displacement; the structure of their songs may still be very similar to that of conspecifics in allopatric populations. It may continue to change until it becomes less recognizable to black-capped chickadees, and at that point, it may be perceived as less of a threat. In other words, the two species may not have co-occurred long enough for the dominant black-capped chickadee to recognize the sympatric mountain chickadee songs as indicative of a lower threat, and for the mountain chickadee to differentiate its song enough to reduce aggression from the dominant species. These small alterations may represent only the beginning stages of adjustment, on the way to increasing the complexity of mountain chickadee songs in regions of overlap with the black-capped chickadees.

Due to the natural segregation of both species based on habitat type, there tend to be small areas of overlap rather than large zones. The existence of these isolated areas may result in independent character shifts within each of the distinct sympatric populations (Grava et al. 2013), that is, there may be a variety of different character shifts. Because chickadees rely on learning for song development, cultural evolution and the rapid emergence of local dialects within sympatric populations of mountain chickadees may limit the ability of black-capped chickadees to recognize and respond to non-local mountain chickadee song variants.

Another possible explanation for why mountain chickadees exhibit character shifts in sympatry without reduced aggression from black-capped chickadees might relate to female mate-choice. Both mountain chickadees and black-capped chickadees are socially monogamous, and songs are thought to have evolved primarily through sexual selection. Female birds tend to prefer long, complex and variable songs, leading to selection pressure on males to lengthen or alter their songs (Catchpole 1980). In black-capped chickadees, females can learn the relative rank of the male based on non-pitch-based cues in vocalizations, and respond more to dominant male songs (Hoeschele et al. 2010). However, Ratcliffe and Otter (1996) suggest that females exhibit reduced response to songs with incorrect internote intervals and flattened within-note frequency ratios in the *fee* note. Additionally, Christie et al. (2003) shows that songs are highly stabilized and that the ability to maintain consistency is a dominance-related characteristic. In regions of co-occurrence, female mountain chickadees occasionally mate with the dominant black-capped chickadee males (Grava et al. 2012a), which could act as selective pressure for the male mountain chickadees to alter their song structures to secure mates. In other words, mountain chickadee males in the presence of dominant black-capped males may alter songs, such as by

adding a song variant in regions of sympatry, to effectively compete for mates with black-capped males, rather than to reduce aggression from black-capped chickadees.

In black-capped chickadees, social hierarchies govern access to resources, as well as social and extra-pair mate choice (Ramsay et al. 2000). Based on the social hierarchies that govern social behaviour, interspecific hierarchies could potentially drive hybridization, if females are choosing dominant males as extra-pair partners regardless of species (Grava et al. 2012a). Both black-capped and mountain chickadees are monogamous and form season-long pair-bonds; however, Grava et al. (2012a) found that in regions of overlap, black-capped males sire the majority of mountain chickadee nestlings through extra-pair copulations, rather than through social pairings (Grava et al. 2012a). This pattern can also be seen in hybrid zones between Carolina chickadees (*Poecile carolinensis*) and black-capped chickadees, where female black-capped chickadees prefer dominant male Carolina chickadees (Bronson et al. 2003; Reudink et al. 2006). As the subordinate species, male mountain chickadees may have reduced expression of a favored dominance trait, leading to mating of the female mountain chickadees with the dominant male black-capped chickadees. This could reduce the potential reproductive output of male mountain chickadee (Grava et al. 2012a; Grava et al. 2013). It would thus be advantageous for the subordinate mountain chickadee to undergo a character shift that could potentially increase attractiveness.

My results do not follow the pattern of character shifts acting to reduce aggression from dominant species, as observed in Asian and European tits, close relatives of North American chickadees. Repeating this study on other black-capped chickadee populations across the shared range may prove informative, as it is possible that this allopatric population of black-capped chickadees in Prince George, BC provided an atypical response. The form of character

displacements in terms of song divergence varies across sympatric populations. Because altered songs vary across populations, this may suggest black-capped chickadees require a period of song learning to recognize local song variants. As such, I would predict that only black-capped chickadees in sympatric populations may exhibit a differentiated response, and that black-capped chickadees may only reduce aggression towards the local mountain chickadee variant. In addition, it may be beneficial to control for black-capped chickadee response to black-capped songs (i.e., conspecific song). Presenting a male black-capped chickadee with both a black-capped song (control) and either an allopatric or sympatric mountain chickadee songs would allow black-capped chickadees to compare between conspecific and heterospecific songs, potentially resulting in greater differentiation between sympatric and allopatric songs. Furthermore, incorporation of Carolina chickadee vocalizations, to which black-capped chickadees in Western Canada have not been exposed, into a playback presented to black-capped chickadees may allow us to interpret whether black-capped chickadees are merely responding to a 'chickadee-like' vocalization, or if they do in fact differentiate between species. Finally, mate choice trials in which female mountain chickadees are presented songs from sympatric and allopatric populations could indicate whether song shifts exhibited by mountain chickadees are driven by sexual selection pressures.

My results demonstrate that mountain chickadee songs with the sympatric song variant do not reduce heterospecific aggression from black-capped chickadees. This finding is particularly interesting as, to my knowledge, within the family Paridae it is the only instance of a character shift being unassociated with reduced aggression. It thus raises interesting questions about the selective pressures leading to the evolution of this song divergence. It is possible that

different selective pressures (e.g. social, sexual) may result in similar evolutionary outcomes in the form of altered songs in sympatric populations.

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APPENDIX A

Table A1. List of all raw data variables obtained from R statistical software, used for principal component analysis. Bolded values represent variables condensed using PCA in my data analysis.

ID	The focal black-capped chickadee
Trial	Trial number (1 or 2)
Allo/sym	Stating whether the trial was an Allopatric or Sympatric playback
First trial	First playback trial (allopatric or sympatric)
Dist_start	Distance of focal bird at beginning of playback
Playback_c_n	Number of calls during the playback
Playback_g_n	Number of gargles during the playback
Playback_c_length	Average length of calls during the playback
Playback_c_dees	Total number of *dees* in a call during the playback
Playback_g_length	Average length of gargles during the playback
Playback_dist_min	Minimum distance from the speaker during the playback
Playback_l_min	Latency to the minimum distance during the playback
playback_0 - 1m	Time spent between 0 and 1 metres of the speaker during playback
Playback_>1-5m	Time spent between 1 and 5 metres of the speaker during playback
Playback_>5-10m	Time spent between 5 and 10 metres of the speaker during playback
Playback_>10-20m	Time spent between 10 and 20 metres of the speaker during playback
Playback_>20m	Time spent greater than 20 metres from the speaker during playback
Playback_s_non-overlapping_n	Total number of songs that don't overlap the stimuli (non-overlapping) during the playback.
Playback_s_non-overlapping_length	Average length of non-overlapping songs during the playback
Playback_s_non-overlapping_amt	Amount of non-overlapping of the stimuli on the focal male songs during the playback
Playback_s_non-overlapping_perc	Percent of non-overlapping of the stimuli on the focal male responses (songs) during the playback
Playback_s_non-overlapping_freq	Frequency of non-overlapping songs during the playback
Playback_s_overlapped_n	Total number of songs overlapped by stimuli during the playback
Playback_s_overlapped_length	Length of songs overlapped by stimuli during the playback
Playback_s_overlapped_amt	Amount of overlap of the stimuli on the focal male songs during the playback
Playback_s_overlapped_perc	Percent of overlap of the stimuli on the focal male responses (songs) during the playback
Playback_s_overlapped_freq	Frequency of the focal male's overlapped song during the playback
Playback_s_overlapping_n	Number of songs that overlap stimuli during the playback
Playback_s_overlapping_length	Length of songs that overlap stimuli during the playback
Playback_s_overlapping_amt	Amount of overlap of the focal male's song on the stimuli during the playback
Playback_s_overlapping_perc	Percent of overlap of the focal male's song on the stimuli during the playback
Playback_s_overlapping_freq	Frequency of the focal male's song when overlapping the stimuli during the playback

Playback_s_total_n	Total number of focal male song responses during the playback
Playback_s_total_length	Total length of focal male song responses during the playback
Playback_s_total_freq	Total frequency of focal male song responses during the playback
Post_c_n	Number of calls during the post period
Post_g_n	Number of gargles during the post period
Post_c_length	Length of calls during the post period
Post_c_dees	Number of dees during the post period
Post_g_length	Length of gargles during the post period
Post_dist_min	Minimum distance to the speaker during the post period
Post_l_min	Latency to the minimum distance during the post period
Post_0-1m	Time spent between 0 and 1 metres of the speaker during post period
Post_>1-5m	Time spent between 1 and 5 metres of the speaker during post period
Post_>5-10m	Time spent between 5 and 10 metres of the speaker during post period
Post_>10-20m	Time spent between 10 and 20 metres of the speaker during post period
Post_>20m	Time spent greater than 20 metres from the speaker during post period
Post_s_non-overlapping_n	Number of songs that don't overlap the stimuli (non-overlapping) during the post period
Post_s_non-overlapping_length	Average length of non-overlapping songs during the post period
Post_s_non-overlapping_amt	Amount of non-overlapping of the focal male's song on the stimuli during the post period
Post_s_non-overlapping_perc	Percent of non-overlapping of the focal male's song on the stimuli during the post period
Post_s_non-overlapping_freq	Frequency of non-overlapping songs during the post period
Post_s_overlapped_n	Number of songs overlapped by stimuli during the post period
Post_s_overlapped_length	Average length of overlapped songs during the post period
Post_s_overlapped_amt	Amount of overlapped songs of the focal male on the stimuli during the post period
Post_s_overlapped_perc	Percent of overlapped of the focal male's song on the stimuli during the post period
Post_s_overlapped_freq	Frequency of overlapped songs during the post period
Post_s_overlapping_n	Number of songs that overlap stimuli during the post period
Post_s_overlapping_length	Average length of overlapping songs during the post period
Post_s_overlapping_amt	Amount of overlap of the focal male's song on the stimuli during the post period
Post_s_overlapping_perc	Percent of overlap of the focal male's song on the stimuli during the post period
Post_s_overlapping_freq	Frequency of overlapping songs during the post period
Post_s_total_n	Total number of focal male song responses during the post period
Post_s_total_length	Total length of focal male song responses during the post period
Post_s_total_freq	Total frequency of focal male song responses during the post period